Genetic Variability in Stress Tolerance and Fitness among Natural Populations of *Steinernema carpocapsae*

Nethi Somasekhar,* Parwinder S. Grewal,*,1 and Michael G. Klein†

*Department of Entomology, Ohio State University, Wooster, Ohio 44691; and †USDA, Agricultural Research Service, Horticultural Insects Research Laboratory, 1680 Madison Avenue, Wooster, Ohio 44691

Received June 15, 2001; accepted October 29, 2001

Genetic variability in stress tolerance (heat, desiccation, and hypoxia) and fitness (virulence and reproduction potential) among natural populations of Steinernema carpocapsae was assessed by estimating phenotypic differences. Significant differences were observed in stress tolerance among populations. Populations isolated from North Carolina showed significantly more stress tolerance than those isolated from Ohio. Significant differences were also observed in populations isolated from the same locality. Survival of infective juveniles after exposure to 40°C for 2 h ranged from 37 to 82%. A threefold difference was observed in infective juvenile survival following exposure to osmotic desiccation or hypoxic condition. Several populations tested were superior to the most widely used strain ('All' strain) in stress tolerance traits, with one population KMD33, being superior to the 'All' strain in all traits. Fitness as expressed by virulence and reproductive potential differed significantly among populations but showed less variability than the stress tolerance traits. All populations tested had a reproductive potential greater than or similar to that of the 'All' strain and most of them caused >60% insect mortality of the wax moth larvae, Galleria mellonella. The high genetic variability in stress tolerance among natural populations suggests the feasibility of using selection for genetic improvement of these © 2002 Elsevier Science (USA)

Key Words: entomopathogenic nematode; natural populations; genetic variability; stress tolerance; fitness.

INTRODUCTION

Entomopathogenic nematodes in the families Steinernematidae and Heterorhabditidae have great promise as biological alternatives to chemicals for the control of soil-inhabiting insect pests (Kaya and Gaugler,

¹ To whom correspondence should be addressed. Fax: (330) 263-3686. E-mail: grewal.4@osu.edu.

1993; Grewal and Georgis, 1998). Their impressive list of attributes including high virulence, ease of mass production, broad host range, and safety (exemption of registration by the U.S. Environmental Protection Agency) has generated a great deal of scientific and commercial interest in these insect-killing nematodes. Despite tremendous advances in research and development, implementation of entomopathogenic nematodes under field conditions still remains hampered by the lack of predictability in efficacy of control (Georgis and Gaugler, 1991; Gaugler et al., 1997; Grewal and Georgis, 1998). Sensitivity to environmental stresses (heat, UV, and desiccation) is one of the key factors attributed to the inconsistencies in the field performance of entomopathogenic nematodes (Kaya, 1990; Georgis and Gaugler, 1991; Gaugler et al., 1997). Genetic improvement has been proposed as a means of improving field efficacy of entomopathogenic nematodes (Gaugler, 1987; Kaya and Gaugler, 1993; Fodor et al., 1994; Burnell and Dowds, 1996). Entomopathogenic nematodes offer several advantages as subjects for genetic improvement including short generation, small genome size, ease of culture and handling, and suitability for inundative applications (Gaugler et al., 1989b; Fodor et al., 1994). Several studies have demonstrated the feasibility of genetic improvement of beneficial traits of entomopathogenic nematodes including host-finding (Gaugler et al., 1989a; Gaugler and Campbell, 1991), virulence (Tomalak, 1994; Peters and Ehlers, 1998), and temperature tolerance (Griffin and Downes, 1994; Grewal et al., 1996).

Selection of proper candidate species, target traits, and provision of adequate genetic variability in base populations are the essential prerequisites for a sound genetic improvement program (Hoy, 1986; Gaugler *et al.*, 1989b; Hastings, 1994). *Steinernema carpocapsae* (Weiser) is an ideal candidate for genetic improvement because it has been extensively field tested against a wide range of insect pests and it possesses superior storage and mass production characteristics (Kaya and Gaugler, 1993; Grewal and Georgis, 1998). Tolerance



to environmental stress and/or enhanced host finding ability that reduces the period of exposure to environmental stress have been proposed as important phenotypic targets for genetic improvement of entomopathogenic nematodes (Gaugler *et al.*, 1989b; Burnell and Dowds, 1996). However, recent findings that *S. carpocapsae* is primarily a surface-adapted ambusher (Campbell and Gaugler, 1993) imply that enhanced tolerance to environmental stresses would be an ideal target for the genetic improvement of field efficacy of *S. carpocapsae*.

Collection and evaluation of wild-type populations from diverse locations are necessary for providing adequate genetic variability in target traits for selection. However, studies on genetic variability for environmental stress tolerance in natural populations of S. carpocapsae are limited. Gaugler et al. (1989b) evaluated the genetic variability among 21 geographical strains of S. carpocapsae for UV tolerance and hostfinding ability. Grewal et al. (1994b) studied the thermal niche breadths for strains of *Steinernema* spp. Since most of the strains tested in both the studies were cultured in the laboratory for several years, they may have lost some of the actual variability. Dramatic changes in biological traits of entomopathogenic nematodes during laboratory adaptation have been demonstrated in several studies (Grewal et al., 1996; Stuart and Gaugler, 1996; Wang and Grewal, 2002). Therefore, it is critical that assessments on natural populations are made using newly field-collected populations. The objectives of the present study were (i) to assess the genetic variability in environmental stress tolerance (heat, desiccation, and hypoxia) and fitness (virulence and reproductive potential) of freshly isolated natural populations of S. carpocapsae, (ii) to identify populations possessing traits superior to those of the most widely used commercial strain ('All' strain), and (iii) to compare populations isolated from different localities. The infective juvenile of entomopathogenic nematode is the only stage that prevails in the soil and is capable of locating and invading a suitable host. Hence, it is most likely that it undergoes natural selection for environmental stress tolerance for successful perpetuation in soil. Consequently, we predicted that there should be considerable variability in the stress tolerance among natural populations of S. carpocapsae.

MATERIALS AND METHODS

Nematode Populations

Fourteen populations recently isolated from diverse locations and a commercial strain ('All' strain) of *S. carpocapsae* were used in this study (Table 1). These populations were confirmed to be *S. carpocapsae* based on infective juvenile length and male tail and spicule

TABLE 1

Locality, Host Insect, and Source of *Steinernema*carpocapsae Populations Used in the Present Study

Population	Locality	Source
All	Georgia	<i>Vitacea polistiformis</i> Harris
KMD-2	Columbus, Ohio	Popillia japonica (Newman)
KMD3	Ashland, Ohio	P. japonica
KMD4	Ashland, Ohio	P. japonica
KMD5	Akron, Ohio	Soil ^a
KMD11	Akron, Ohio	Soil
KMD14	Akron, Ohio	Soil
KMD7	Wooster, Ohio	Soil
KMD18	Broadview Heights, Ohio	Soil
KMD38	Portsmouth, Ohio	Soil
KMD52	Portsmouth, Ohio	Soil
KMD28	Lenoir, North Carolina	Soil
KMD30	Lenoir, North Carolina	Soil
KMD33	Lenoir, North Carolina	Soil
KMD26	Chantilly, Virginia	Soil

^a Nematodes were isolated from soil using last instar larvae of *Galleria mellonella* as bait.

morphology (P. S. Grewal, unpublished data). All newly isolated populations were cultured once in the wax moth *Galleria mellonella* L. larvae at 25°C following isolation as described by Kaya and Stock (1997) and stored in liquid nitrogen as described by Curran *et al.* (1992) to prevent changes in field-adapted traits due to frequent culturing in the laboratory. 'All' strain was maintained in the laboratory by culturing in wax moth larvae at 1- to 2-month intervals. The nematodes cryopreserved in the liquid nitrogen were thawed in Ringer's solution for 24 h. All the populations were cultured twice in the wax moth larvae prior to performing the bioassays. Infective juveniles emerging within the first 2–3 days from the wax moth larvae were used in the bioassays.

Stress Tolerance

Heat. Heat tolerance of infective juveniles of different populations was assessed by exposure to 40°C for 2 h in incubator. One thousand infective juveniles in 1 ml of water were placed in a well of a 24-well plate. One such well was maintained for each population in each well plate. Six replicates were prepared for each population. After exposure to 40°C , the plates were incubated at 25°C for 24 h for recovery of the nematodes from heat shock. Six additional plates constantly maintained at 25°C for each population were used as control. The number of dead and live nematodes was counted by taking three 50- μ l samples from each well, and percentage survival was calculated. The inactive nematodes were considered dead if they did not respond to probing with a steel probe. The bioassay was

performed two times using a different generation of infective juveniles and six replications each time.

Desiccation. Desiccation tolerance of the nematode populations was evaluated by dehydrating the infective juveniles in glycerol solution as described by Glazer and Salame (2000). Briefly, 1000 infective juveniles in 0.5 ml of water were mixed with 0.5 ml of 80% (v/v) glycerol solution to obtain a final glycerol concentration of 40% (v/v). The nematodes mixed in glycerol were placed in a well of a 24-well plate. One such well was maintained for each population in each well plate. Six replicates were prepared for each population. After incubation at 25°C for 96 h, the nematodes were rehydrated in 10 ml of water. Six additional plates containing nematodes in water at the same concentration were used as control for each population. Nematode survival was determined 24 h after rehydration at 25°C as described above. The bioassay was performed two times using a different generation of infective juveniles and six replications each time.

Hypoxia. Tolerance to hypoxia was assayed by storing the infective juveniles in 0.5 ml of water in 0.5-ml air-tight Eppendorf tubes. Ten thousand infective juveniles were held in each tube at 25°C with the lids tightly closed. Six tubes were prepared for each population. After 10 days, the nematodes were transferred to 10-cm-diameter petri dishes containing 9 ml of water and incubated at 25°C for 24 h for the recovery of the nematodes from hypoxia. Six 5-cm-diameter petri dishes containing nematodes at the same concentration were used as control and held at 25°C. Nematode survival was determined as described above. The bioassay was performed two times using a different generation of infective juveniles and six replications each time.

Fitness

Virulence. Virulence of infective juveniles against last instar larvae of *G. mellonella* was assessed using the one-on-one sand-well bioassay described by Grewal et al. (1999). Four 24-well plates were set up for each nematode population and an additional four plates were set up without nematodes to assess control insect mortality. The number of dead insect larvae in each plate was recorded after 48 and 72 h, and percentage mortality was calculated. The bioassay was performed two times using a different generation of infective juveniles and four replications each time.

Reproductive potential. Reproductive potential of the nematode populations was determined by infecting last instar larvae of the wax moth individually with 100 infective juveniles on a filter paper in wells of a 24-well plate at 25°C. After 72 h, the infected wax moth cadavers were transferred to White traps (White, 1925), placing one cadaver in each trap. Five such traps were maintained for each nematode population.

The total number of infective juveniles produced per wax moth larvae at 25°C was recorded 20 days after initial infection. The experiment was repeated two times using a different generation of infective juveniles and five replications each time.

Statistical Analyses

Percentage data were normalized using arcsine transformation and subjected to analysis of variance (ANOVA) using Statistica release 5.5 (Statsoft, 1999). Data from two experiments were pooled for analysis as the interaction between experiment and nematode population was not significant (P > 0.05) for all traits. The differences among populations were compared using Tukey's test and linear contrasts. Differences between the control and treatment within each population were compared using t test. $P \le 0.05$ was considered significant.

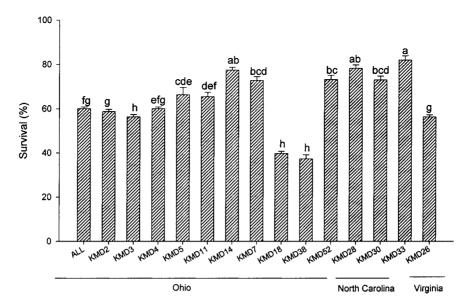
RESULTS

The interaction between experiment and nematode population was not significant for any trait [F=0.16; df=14, 150; P=0.999 (heat tolerance); F=0.08; df=14, 150; P=0.999 (desiccation tolerance); F=0.51; df=14, 150; P=0.923 (hypoxia tolerance); F=0.02; df=14, 150; P=1.00 (virulence); F=0.01; df=14, 120; P=1.00 (reproductive potential)]. These data indicate that the performance of different generations of infective juveniles used in two experiments was not significantly different. Consequently, results based on the data pooled from two experiments are presented here.

Stress Tolerance

Heat. Populations of *S. carpocapsae* differed significantly (F=68.81, df=14, 154; P<0.0001) to heat tolerance (Fig. 1). Survival of infective juveniles exposed to 40° C for 2 h varied between 37 and 82% among populations. No mortality was observed in controls. Nine populations had over 60% survival and two had less than 40% survival. Populations isolated from North Carolina were significantly more heat tolerant than populations isolated from Ohio (F=7.36; df=1, 154; P=0.007). Seven populations were significantly more (P<0.05) heat tolerant than the 'All' strain. Significant differences were observed in heat tolerance among populations isolated from the same locality in both Ohio (KMD11, KMD14, KMD38, KMD52) and North Carolina (KMD30, KMD33).

Desiccation. Desiccation survival differed significantly (F = 177.78; df = 14, 154; P < 0.001) among the 15 populations of *S. carpocapsae* (Fig. 2). No mortality was observed in controls. Survival of infective juveniles following osmotic desiccation for 96 h varied

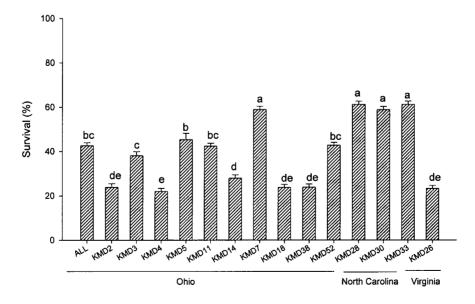


Steinernema carpocapsae populations

FIG. 1. Mean survival (% + SE) of infective juveniles of *Steinernema carpocapsae* populations after incubation at 40°C for 2 h. Bars with same letter(s) do not differ significantly according to Tukey's test ($P \le 0.05$).

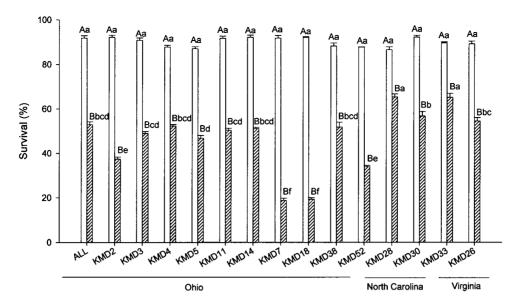
between 22 and 61%. Only two populations had over 60% survival and five had less than 25% survival. Populations isolated from North Carolina were significantly more desiccation tolerant than the populations isolated from Ohio (F=541.78; df=1, 154; P<0.001). Four populations were significantly more (P<0.05) desiccation tolerant than the 'All' strain. Significant differences were observed in desiccation tolerance among populations isolated from the same locality (KMD11, KMD14, KMD38, KMD52).

Hypoxia. Survival of *S. carpocapsae* populations under hypoxic conditions also differed significantly $(F=102.82;\ df=14,\ 154;\ P<0.0001)$ (Fig. 3). Survival of infective juveniles was significantly greater in control compared to the hypoxia treatment for all populations (P>0.05). No significant differences in control mortality were observed among populations (P>0.05). Survival of infective juveniles following exposure to hypoxic condition for 10 days varied between 18 and 65% among populations, with KMD28



Steinernema carpocapsae populations

FIG. 2. Mean survival (% + SE) of infective juveniles of *Steinernema carpocapsae* populations after desiccation in 40% glycerol for 96 h. Bars with same letter(s) do not differ significantly according to Tukey's test ($P \le 0.05$).



Steinemema carpocapsae populations

FIG. 3. Mean survival (% + SE) of infective juveniles of *Steinernema carpocapsae* populations exposed to (\square) hypoxia for 10 days. Bars with same lower case letter(s) do not differ significantly among populations according to Tukey's test ($P \le 0.05$) and bars within each population with same upper case letter do not differ significantly according to t test ($P \le 0.05$).

and KMD33 showing highest survival and KMD7 and KMD18 showing the least. Two populations (KMD7 and KMD18) had less than 25% survival and two populations (KMD28 and KMD33) had more than 60% survival. Populations isolated from North Carolina were significantly more tolerant to hypoxia than the populations isolated from Ohio (F=784.61; df=1, 154; P<0.0001). Two populations were significantly more (P<0.05) tolerant to hypoxia than the 'All' strain. Significant differences were observed in hypoxia tolerance among populations isolated from the same locality in both Ohio (KMD38, KMD52) and North Carolina (KMD28, KMD30, KMD33).

Fitness

Virulence. Virulence differed significantly among populations of S. carpocapsae [F = 59.48; df = 15,105; P < 0.0001 (48 h); F = 32.50; df = 15, 105; P < 0.00010.0001 (72 h)] (Fig. 4). Mortality of wax moth larvae among populations varied between 22 and 76% and between 42 and 79% at 48 and 72 h after inoculation of nematodes, respectively. Mortality in control was less than 6%. Nine populations caused over 60% mortality of wax moth larvae. Although after 48 h, KMD33 caused significantly greater mortality (P < 0.05) than all other populations except KMD11, after 72 h there was no significant difference (P > 0.05) in mortality between KMD33 and five other populations including 'All' strain. Significant differences were observed in virulence among populations isolated from the same locality in both Ohio (KMD11, KMD14) and North Carolina (KMD30, KMD33).

Reproductive potential. There were significant differences in progeny production among populations of S. carpocapsae (F=8.94; df=14, 126; P<0.0001) (Fig. 5). Four populations produced significantly greater progeny than the 'All' strain (P<0.05). The number of infective juveniles produced per insect varied from 111×10^3 to 169×10^3 . Significant differences were observed in reproduction among populations isolated from the same locality (KMD11, KMD14).

DISCUSSION

Our results demonstrate large phenotypic differences in stress tolerance (heat, desiccation, and hypoxia) among natural populations of *S. carpocapsae*. Greater variance among populations and similar levels of phenotypic expression in bioassays performed with different generations of infective juveniles indicate a strong genetic component underlying this phenotypic variation.

Genetic improvement of environmental tolerance of entomopathogenic nematodes would improve their field performance and enable their use under suboptimal conditions. When aiming for nematode strains with improved traits, first it is preferable to look for preadapted wild types as such genotypes are likely to be more stable. The superior performance of the population KMD33 over the most widely used 'All' strain of *S. carpocapsae* indicates the possibility of developing this population as a commercial strain. Therefore, the virulence against target pests, field efficacy, mass production characteristics, and stability of the superior

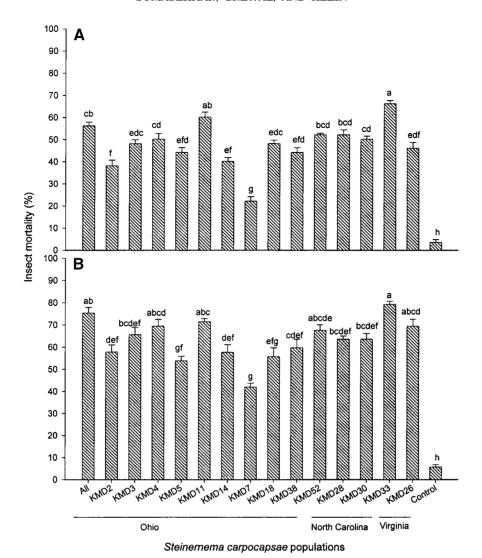


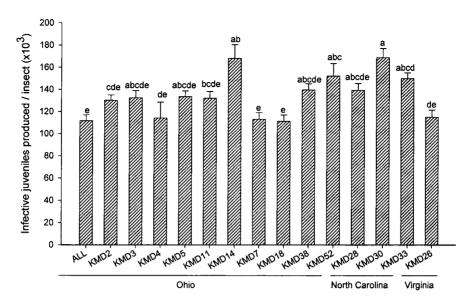
FIG. 4. Mean mortality (% + SE) of *Galleria mellonella* larvae caused by infective juveniles of *Steinernema carpocapsae* populations in the one-on-one sand-well bioassay at 48 h (A) and 72 h (B) after inoculation. Bars with same letter(s) do not differ significantly according to Tukey's test ($P \le 0.05$).

traits of this population need to be examined in future studies.

If the wild-type populations with the desired level of competence are not available, but there is a high variability for target traits in the wild-type populations, then the next ideal approach for genetic improvement would be initiating a systematic selection program by constructing a genetically diverse foundation line. Significant differences were observed in heat tolerance not only among populations isolated from different geographical regions but also among those isolated from the same locality. P. S. Grewal and X. Wang (unpublished) also observed significant differences among natural populations of *Heterorhabditis bacteriophora* Poinar isolated from a single field. A more than twofold difference was observed between the most and the least heat-tolerant population and several populations

were superior to the 'All' strain, suggesting that selection would be an efficient approach for improving this trait. A high level of heritability for heat tolerance (Glazer *et al.*, 1991) and response to selection for temperature tolerance (Griffin and Downes, 1994; Grewal *et al.*, 1996) has been demonstrated in *Heterorhabditis* spp. A rapid response to selection for high and low temperature tolerance was also demonstrated in steinernematids (Grewal *et al.*, 1996; Jagdale and Gordon, 1998).

Tolerance to desiccation and hypoxia also varied significantly among populations of *S. carpocapsae*. Significant differences were observed even among the populations isolated from the same locality. A threefold difference between the least and the most tolerant population and the superiority of several populations to the most widely used 'All' strain suggest that selec-



Steinernema carpocapsae populations

FIG. 5. Mean numbers of infective juveniles (+SE) produced per insect larva by populations of *Steinernema carpocapsae* at 25°C. Bars with same letter(s) do not differ significantly according to Tukey's test ($P \le 0.05$).

tive breeding for the genetic improvement of these traits would be worthwhile pursuing. Glazer *et al.* (1991) demonstrated a low heritability for desiccation tolerance in *H. bacteriophora* using inbred lines. Therefore, mutagenesis has been used to develop desiccation-tolerant mutants in this species (O' Leary and Burnell, 1997). However, information on heritability and response to selection for desiccation tolerance in steinernematids is limited and hence needs to be a focus of future studies. Although tolerance to hypoxic conditions is relatively less critical compared with temperature, UV, and desiccation in improving the field performance of entomopathogenic nematodes, it is important in improving the survival of infective juveniles in water-logged soils and liquid formulations.

Wild-type populations may possess novel traits that offer advantages in biological control. However, if the populations do not possess a desirable level of fitness such as virulence and reproductive potential, the improvements in biocontrol efficacy are minimal. Our results demonstrate that all populations tested in this study have a reproductive potential higher than or similar to that of the 'All' strain, with most of them causing >60% insect mortality in the one-on-one virulence bioassay. These data suggest that the fitness of these populations is comparable to that of the commercial strain. Although significant differences were observed in virulence and reproductive potential among populations, variability in these traits is relatively low (less than twofold difference among populations) compared to the stress tolerance traits. This may be because the virulence and reproductive potential were evaluated against a highly susceptible laboratory host under ideal conditions rather than a natural host under field conditions. Interestingly, "All" strain was not greatly superior to the newly isolated populations in the fitness tests even though it has been passed through the laboratory host *G. mellonella* many times over the years.

The ability to tolerate environmental stresses is thought to be related to the climatic conditions of the locality from where the nematode population was isolated (Molyneux, 1986). However, our results demonstrate significant differences in stress tolerance among populations isolated from the same locality. Possibly differences in the microenvironment in their soil niche may be playing an important role in selection for stress tolerance in entomopathogenic nematodes as they have a patchy distribution in soil and a limited ability to disperse over long distances (Stuart and Gaugler, 1994; Campbell et al., 1996). Significant genetic differences among populations isolated from a single field have been found in *H. marelatus* Liu and Berry and *S.* feltiae (Filipjev) (Blouin et al., 1999; Hominick et al., 1999). P. S. Grewal and X. Wang (unpublished) observed significant differences in stress tolerance among populations of *H. bacteriophora* isolated from the same field. These studies along with our results suggest that several genetically distinct populations of the same species of entomopathogenic nematodes may occur in the same field. Coexistence of conspecific populations possessing varying degrees of adaptations may be a strategy for reducing the intraspecific competition and insuring the perpetuation of species against environmental extremes.

ACKNOWLEDGMENT

This research was funded by USDA CREES Grant No. 00-35302-9336 to P. S. Grewal.

REFERENCES

- Abbott, W. S. 1925. A method of computing the effectiveness of an insecticide. *J. Econ. Entomol.* **18**, 265–276.
- Blouin, M. S., Liu, J., and Berry, R. E. 1999. Life cycle variation and genetic structure of nematode populations. *Heredity* **83**, 253–259.
- Burnell, A. M., and Dowds, B. C. A. 1996. The genetic improvement of entomopathogenic nematodes and their symbiotic bacteria: Phenotypic targets, genetic limitations and an assessment of possible hazards. *Biocontr. Sci. Technol.* **6**, 435–447.
- Campbell, J. F., and Gaugler, R. 1993. Nictation behavior and its ecological implications in the host search strategies of entomopathogenic nematodes (Heterorhabditidae and Steinernematidae). *Behaviour* **126**, 156–169.
- Campbell, J. F., Lewis, E. E., Yoder, F., and Gaugler, R. 1996. Entomopathogenic nematode (Heterorhabditidae and Steinerne-matidae) spatial distribution in turfgrass. *Parasitology* 113, 473–482.
- Curran, J., Gilbert, C., and Butler, K. 1992. Routine cryopreservation of *Steinernema* and *Heterorhabditis* spp. *J. Nematol.* **24**, 1–3.
- Fodor, A., Marton, P., Szallas, E., and Vellai, T. 1994. Prospects for the genetic improvement/analysis of entomopathogenic nematodes *In* "Genetics of Entomopathogenic Nematode–Bacterium Complexes" (A. M. Burnell, R. U. Ehlers, and J. P. Masson, Eds.), pp. 101–119. European Commission Publication EUR 15681 EN, Luxembourg.
- Gaugler, R. 1987. Entomogenous nematodes and their prospects for genetic improvement. *In* "Biotechnological Advances in Invertebrate Pathology and Cell Culture" (K. Maramorosch, Ed.), pp. 457–484. Academic Press, New York.
- Gaugler, R. and Campbell, J. F. 1991. Selection for enhanced host finding of scarab larvae (Coleoptera: Scarabaeidae) in an entomopathogenic nematode. *Environ. Entomol.* **20**, 700–706.
- Gaugler, R., Campbell, J. F., and McGuire, T. R. 1989a. Genetic selection for host finding in *Steinernema feltiae. J. Invertebr. Pathol.* **54**, 363–372.
- Gaugler, R., Lewis, E., and Stuart, R. J. 1997. Ecology in the service of biological control: The case of entomopathogenic nematodes. *Oecologia* 109, 483–489
- Gaugler, R., McGuire, T., and Campbell, J. F. 1989b. Genetic variability among strains of the entomopathogenic nematode *Steinernema feltiae*. *J. Nematol.* **21**, 247–253.
- Georgis, R. and Gaugler, R. 1991. Predictability in biological control using entomopathogenic nematodes. *J. Econ. Entomol.* **3,** 713–720.
- Glazer, I., and Salame, L. 2000. Osmotic survival of the entomopathogenic nematode Steinernema carpocapsae. Biol. Control 18, 251–257.
- Glazer, I., Gaugler, R., and Segal, D. 1991. Genetics of the nematode *Heterorhabditis bacteriophora* strain HP88: The diversity of beneficial traits. *J. Nematol.* **23**, 324–333.
- Grewal, P. S. and Georgis, R. 1998. Entomopathogenic nematodes. *In* "Methods in Biotechnology," Vol. 5, "Biopesticides: Use and Delivery" (F. R. Hall and J. J. Menn, Eds.), pp 271–299. Humana Press, Totowa, NJ.
- Grewal, P. S., Converse, V., and Georgis, R. 1999. Influence of production and bioassay methods on virulence of two ambush foragers (Nematoda: Steinernematidae). *J. Invertebr. Pathol.* **73**, 40–44.
- Grewal, P. S., Gaugler, R., and Shupe, C. 1996. Rapid evolution of thermal sensitivity of entomopathogenic nematodes in response to selection at temperature extremes. *J. Invertebr. Pathol.* **68**, 65–73.

- Grewal, P. S., Lewis, E. E., Gaugler, R., and Campbell, J. F. 1994a.
 Host finding behavior as a predictor of foraging strategy in entomopathogenic nematodes. *Parasitology* 108, 207–215.
- Grewal, P. S., Selvan, S., and Gaugler, R. 1994b. Thermal adaptation of entomopathogenic nematodes: Niche breadth for infection, establishment, and reproduction. *J. Therm. Biol.* **19**, 245–253.
- Griffin, C. T., and Downes, M. J. 1994. Selection of *Heterorhabditis* sp. for improved infectivity at low temperatures. *In* "Genetics of Entomopathogenic Nematode–Bacterium Complexes" (A. M. Burnell, R. U. Ehlers, and J. P. Masson, Eds.), pp. 143–151. European Commission Publication EUR 15681 EN, Luxembourg.
- Hastings, I. M. 1994. Introduction to quantitative genetics: Inbreeding, heritability estimates and artificial selection. *In* "Genetics of Entomopathogenic Nematode–Bacterium Complexes" (A. M. Burnell, R. U. Ehlers, and J. P. Masson, Eds.), pp. 120–128. European Commission Publication EUR 15681 EN, Luxembourg.
- Hominick, W. M., Hunt, D. J., Reid, A., Briscoe, B. R., and Bohan, D. A. 1999. Biosystematics, phylogeny, and population genetics of entomopathogenic nematodes. *In* "Taxonomy, Phylogeny and Gnotobiological Studies of Entomopathogenic Nematode Bacterium Complexes" (N. Boemare, P. Richardson, and F. Coudert, Eds.), pp. 45–53. European Commission Publication COST819, Brussels.
- Hoy, M. A. 1986. Use of genetic improvement in biological control. Agric. Ecosys. Environ. 15, 109–110.
- Jagdale, G. B., and Gordon, R. 1998. Effect of propagation temperatures on temperature tolerance of entomopathogenic nematodes. Fundam. Appl. Nematol. 21, 177–183.
- Kaya, H. K. 1990. Soil ecology *In* "Entomopathogenic Nematodes in Biological Control" (R. Gaugler and H. K. Kaya, Eds.), pp. 93–115. CRC Press, Boca Raton, FL.
- Kaya, H. K., and Gaugler, R. 1993. Entomopathogenic nematodes. Annu. Rev. Entomol. 38, 181–206.
- Kaya, H. K., and Stock, S. P. 1997. Techniques in insect nematology. In "Manual of Techniques in Insect Pathology" (L. A. Lacey, Ed.), pp. 281–324. Academic Press, London.
- Molyneux, A. S. 1986. *Heterorhabditis* spp. and *Steinernema* (= *Neoaplectana*) spp.: Temperature, and aspects of behaviour and infectivity. *Exp. Parasitol.* **62**, 169–180.
- O'Leary, S. A., and Burnell, A. M. 1997. The isolation of mutants of *Heterorhabditis megidis* (Strain UK211) with increased desiccation tolerance. *Fundam. Appl. Nematol.* **20**, 197–205.
- Peters, A., and Ehlers, R. U. 1998. Evaluation and selection for enhanced nematode pathogenicity against *Tipula* spp. *In* "Pathogenicity of Entomopathogenic Nematodes versus Insect Defense Mechanisms: Impact on Selection of Virulent Strains" (N. Simoes, N. Boemare, and R. U. Ehlers, Eds.), pp. 225–241. European Commission Publication COST819, Brussels.
- Statsoft, Inc. 1999. Statistica for Windows release 5.5 (Computer program manual), Tulsa, OK.
- Stuart, R. J., and Gaugler, R. 1994. Patchiness in populations of entomopathogenic nematodes. *J. Invertebr. Pathol.* **64**, 39–45.
- Stuart, R. J., and Gaugler, R. 1996. Genetic adaptation and founder effect in laboratory populations of the entomopathogenic nematodes *Steinernema glaseri*. Can. J. Zool. 74, 164–170.
- Tomalak, M. 1994. Selective breeding of *Steinernema feltiae* (Filipjev) (Nematoda, Steinernematidae) for improved efficacy in control of a mushroom fly *Lycoriella solani* Winnertz (Diptera, Sciaridae). *Biocontr. Sci. Technol.* **4,** 187–198.
- Wang, X. and Grewal, P. S. 2002. Rapid genetic deterioration of environmental tolerance and reproductive potential of an entomopathogenic nematode during laboratory maintenance. *Biol. Control* 23, 71–78.
- White, G. F. 1927. A method for obtaining infective nematode larvae from cultures. *Science* **66**, 302–303.